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Winter Fungi Of The Apple

WINTER FUNGI OF THE APPLE

BY

FRANCES JEAN MacINNES

THESIS

FOR THE

DEGREE OF BACHELOR OF SCIENCE

IN

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UNIVERSITY OF ILLINOIS

May 25, 1916

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Frances Jean MacInnes

ENTITLED Winter Fungi of the Apple

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science

F L Stevens

Instructor in Charge

APPROVED: Wm. Trelease

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BROWN ROT OF APPLES

The name "Brown Rot" as applied to the rot caused by Sclerotina fructigena, or Monilia, as it appears on its various hosts, is not particularly descriptive, for there are other fungi which cause a spot or rot which is just as characteristically brown. It is often called Ripe Rot, Fruit Rot, or Plum Fruit Rot, Plum Rot, Peach Rot, or Peach Blight, when referring to its particular hosts. "Brown Rot", however, is the name most commonly employed in literature.[1]

The scientific names have been as numerous as the common name. The earliest is Torula fructigena, given by Persoon in 1796.[2] Later he changed it to Acrosporium fructigena. Since then it has been Oidium fructigena Lk., Oidium laxum Ehr., Oidium wallrothii Thüm., and Oospora fructigena Waltr. Persoon himself finally referred the fungus to the genus Monilia where Saccardo places it - in the Fungi Imperfecti. In 1893 Schröter surmised the ascomycetous nature of the fungus and placed it in the genus Sclerotinia. Norton[5] in 1902 found the apothecia arising from the sclerotia and was able to produce typical Monilia from the ascospores. Thus its identity as Sclerotinia was definitely established.

There has also been great dificulty in differentiating between S. fructigena and S. cinerea. They will both, when artificially inoculated, grow on either pomaceous or drupace-

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ous hosts. Thus there is no simple means of separating them. Quaintance[3] in 1900 reported "Monilia fructigena" as occuring in the United States on stone fruits in particular and to a much less extent on pomaceous, but says (page 247) "the parasite is in all cases the same". Bartram[6] makes a summary of the conclusions that have been arrived at by those who have worked on these fungi from that time to this. The distinctions are as follows: the conidia of S. fructigena are larger than those of S. cinerea, the conidial tufts are brownish yellow and large, the shape of the conidia is ellipsoidal, it occurs in nature on pomes, and the ascospores are pointed and large. on the other hand S. cinerea has smaller conidia, they are more or less rounded, the tufts are always ash grey and small, and it is in nature found on stone fruits. It has no disjunctors between the conidia and the ascospores are rounded and smaller than those of S. fructigena. Ewert[7] also found a difference in that the conidia of S. cinerea were capable of living over winter while those of S. fructigena usually lose their power to germinate early in the winter. Bartram also reports Methany[8] and Jehle[9] as concluding that the most common form in the United States is S. cinerea. Reade[10] in New York found it to be S. fructigena as did Pollock[11] in Michigan. Conel[12] in Urbana, Illinois, found that the fungus was nearer the description of S. cinerea than to that of S. fructigena. Bartram himself finds that the commonest form of Brown Rot of stone fruits in Vermont is S. cinerea. There are no data given as to which is

found on apples other than the generalization that S. fructigena is in nature on pomaceous fruits.

The blossoms and twigs of the peach, plum, and cherry are reported as attacked by the fungus but no definite reference has been found to the effect that apples are so attacked.[1,3,13,14] The fruits of the apple, however, are very characteristically affected and quite differently from the plum and peach. The plum may be entirely rotted inside and show no external evidence until the tufts of spores appear. The peach shows, immediatly, a more or less circular brown spot which enlarges rapidly until the whole fruit is infected and this soon becomes covered with an ash grey mould. Both the plum and peach may become "mummified" the former retaining clumps of spores on the surface, the latter losing the spores but retaining the internal mycelium.[17]

On the apple the fungus is preceded by a brown decayed patch on which greyish white tufts of spores appear. These are generally in concentric rings[17] remaining so in the mummified fruit. This is not true in the case of the plum and peach. As im the peach the decay continues until the whole fruit is covered. In many cases the diseased fruits do not fall but remain on the trees until spring, when either the old conidia or those of a new crop are ready to infect the new fruit. In some instances when the apples are attacked no conidia are formed until the following season.[15] In this case the skin becomes hard and black. This is perhaps the sclerotial stage.

About the earliest mention of Brown Rot as a distinctive

disease seems to have been in Europe in 1880 by Von Thumen.[1] It was considered most destructive but according to later writers is not as serious now. Frank[16] mentions it as occurring on plums, cherries, apricots, peaches, apples, and pears. In the United States it was first described by Peck in 1881, and has been investigated by many since then. It is particularly destructive to stone fruits. Pomaceous fruits are subject to attack but the loss is not as great as in Europe. On the plum and peach it is reported on the eastern[6] and western[1] coasts and as far south as Georgia[3], showing its general distribution. On the apple it is not destructive in any part of the country, although the apple is well known as one of its hosts.

It is the Monilia stage with the summer spore, which causes the rot in all cases. It was not until recently (1902) that the perfect stage was found.[5] The summer spores are borne on the ends of the hyphal threads. The drawings (Figs. D and E) show the mode of branching and also the shape and size of spores as compared with the hyphae. The end spore is always the youngest, as the new spore forms at the distal end and not by splitting off from the hypha. The branches are formed by the end spore forming an angle and a new chain growing at such point.

The ascigerous stage appears about the time the peach blossoms are opening - April 1 to 27th.[5-18] It seems to be only on two-year-old mummies which have been in moist places in or on top of the soil. There are usually several on each fruit. The sinuous stalk of the apothecium is from .5 to 1.5 cm. long, and from .3 to 1.5 mm. thick. This enlarges into the cup shaped

tube. In this the asci are formed. These contain eight egg-shaped spores each. From these spores typical Monilia spores may be grown. This ascus stage is not necessary for the wintering over of the fungus. The hyphae are capable of withstanding almost any amount of cold when in the mummy and the conidia in may cases do not lose the power of germination even under very severe conditions.[6]

As Monilia on apples is not serious in this country, there is no need at present of any special treatment. The spraying done for other diseases prevents any dangerous infection. From my own inoculations I was unable to produce a rot unless the skin was punctured. This would probably indicate that a serious epidemic is not to be feared. Orchard sanitation is the only precaution necessary. All mummy fruits and any infected twigs should be carefully disposed of.

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EXPLANATION OF PLATE

A, B, C, - Forms of mycelium found in the tissue of the apple, showing method of branching

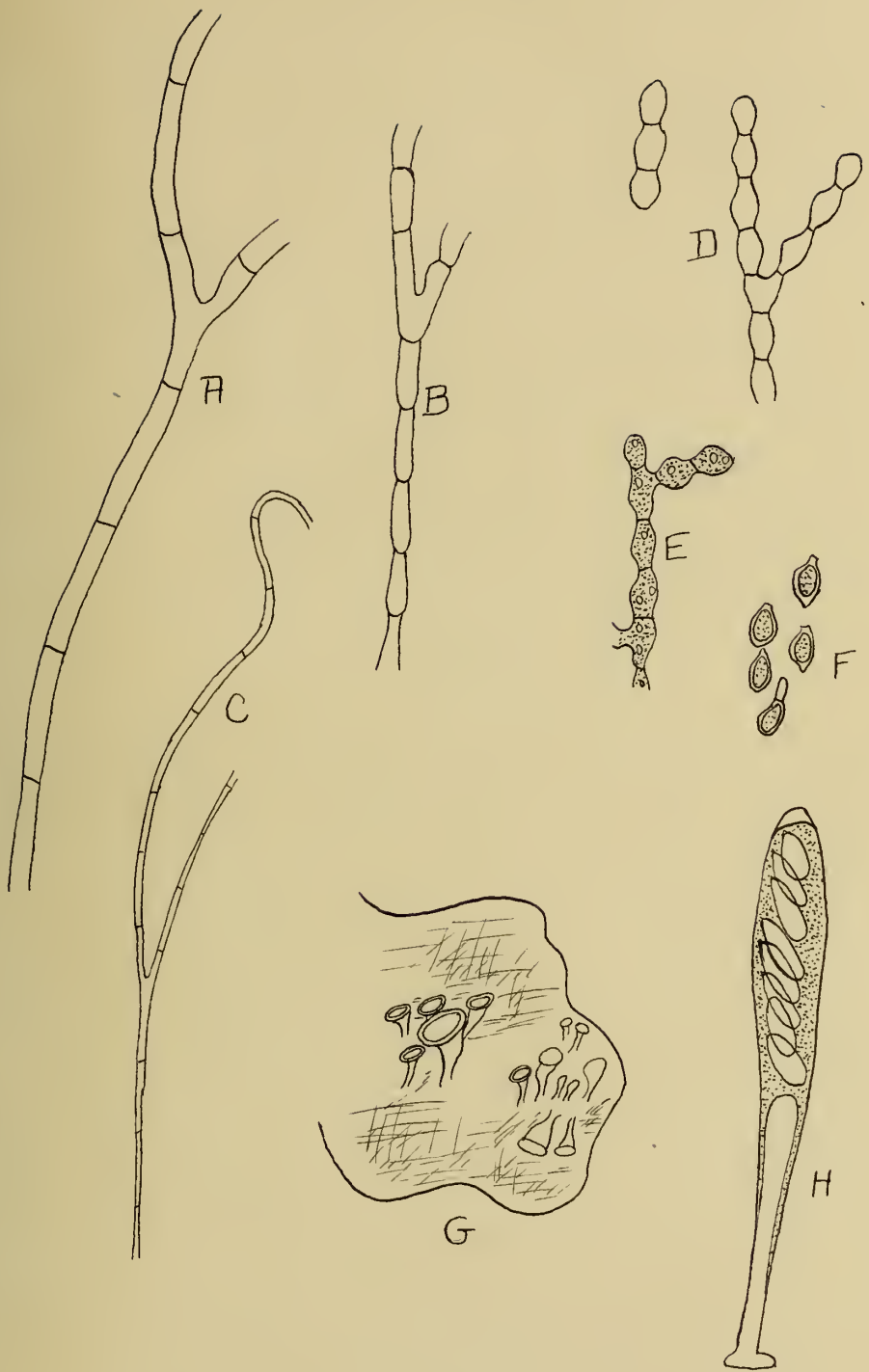
D - Chains of spores showing method of branching (from Prune culture)

E - Chain of spores showing granular appearance (from prune culture)

F - Spores showing lemon shape

G - Apothecium on apple mummy - after Sorauer

H - Ascus and ascospores - after Sorauer



Sclerotinia Fructigena on Apple

AN ALTERNARIA IN APPLE SPOTS

Introduction

The twenty-five apples on which this work is based came from Harristown, Illinois, early in July, and therefore were not nearly mature. The spots were in various stages of development, making it possible to study the probable progress of the disease. Nothing is known of the conditions under which they grew, nor of the time the infection started.

Description

There is no apple disease which causes a more characteristic spot on the fruit than this one. It is striking in its early stages, due to the decided color change; and in the later ones to the distinct margin as well as the darkened skin and tissue. The fruit is not rotted or softened. The outer skin of the diseased portion is tough like leather, and is difficult to cut through. Only a few millimeters beneath the skin are injured. The disease is not destructive to a large part of the fruit, but it is very unsightly, and would decrease salability to a great degree.

Apple No. 1 (See Table I) was considered to be in the earliest stage of the disease. An idea of its condition may be gotten from Plate II, No. 1. There was no softening of the tissue or change in the size or shape of the fruit. The color was the only indication of any disease. The apple, which was clear green

TABLE I

TABLE I

No. of Apple	Description of Spot	Size of Spot
I	Very early stage, color changed from green to yellow, mottled more or less uniformly with red. No change in texture or shape.	1.5 x 1.5cm. (colored spo
2	Slightly darker than (1), probably later stage mottled with both red and brown on the yellow ground. No change in shape or texture.	1.5 x 2.0cm. (colored spo
3	Like above but with a small, irregularly shaped, shiny, hard, reddish brown spot in center. Shape and texture of fruit the same.	2mm x 2mm. (dark spot)
4	Fruit prematurely colored over one third the surface (red and yellow), spots (reddish brown) larger and more numerous.	2mm x 5mm. (dark spot)
5	Irregular brown spots as in (4). Color of fruit not changed.	5mm x 1cm.
6	As in (5) but brown spots more depressed and larger.	5mm. x 1cm.
7	Color of spots darker, slightly depressed as in (5). Slightly reddened tint beyond.	3cm. x 2cm.
8	Spots much darker, slightly depressed, red border quite distinct.	5mm. x 1mm.
9	As in (8) but slightly larger spot. Slightly depressed.	5mm. x 5mm.
10	One large spot, dark brown, surface flattened. Bright red outline.	1cm. x 2cm.
11	As in (10) but margin depressed, red border radiating over a third of the surface.	3cm. x 2cm.
12	Spot very dark, almost black, outline very distinct, red brilliant.	2.5 x 2cm.
13	Spot more regular in outline than (12)	3cm x 3cm

TABLE I

Mycelium	Description of Cross Section	Depth of Disease	Fungus found
None found	No sign of disease in the tissue	---	Aspergellis
None found	No sign of disease in the tissue	---	Alternaria
Distinct	Very slight sign of browned tissue under spot	.25mm.	Alternaria
Distinct	Dark brown tissue beneath spot	.50mm.	Alternaria
Distinct	Loose celled brown tissue	2mm.	Alternaria
Distinct	Brown under the spot, sunken	.5-2mm	Alternaria
Distinct	Brown, depressed irregularly	.5-1mm.	Alternaria
Distinct	Dark brown, cells collapsed under spot	.5 mm.	Alternaria
Distinct	Dark brown, irregularly sunken	.5mm.	Alternaria
Distinct	Dark brown, irregularly sunken	1-1.5mm.	Alternaria
Distinct	Dark brown, dry hard tissue margin depressed	1mm.	Alternaria
Distinct	Very dark, tissue dry and hard	3mm.	Alternaria
Distinct	Very dark, edges depressed	3mm.	Alternaria

and perfectly healthy otherwise, had a spot about 2 cm. in diameter. This part was a delicate yellow mottled with red, and apparently did not change in size or color in the two months it was observed.

The next stage (No. 2) was slightly darker than the first, and from this a culture of Alternaria was readily obtained, although the mycelium was not abundant in the tissue. The fruit in this case was yellow, and the spot slightly darker, mottled with both red and brown. The shape and texture appeared the same as in the rest of the fruit.

The same characteristics as in the first two appeared also in No. 3, but in the center was a slightly sunken brown spot with a distinct border, and radiating around this was the yellow tissue, mottled with red. The brown spot was irregular in shape, (See Plate II, No. III) shiny, hard, and from 2 to 4 cm. in diameter.

In the more advanced stages the red color usually increased. There was still the yellow color mottled or striped with red, or the green blushed with red and the irregular brown spots in the center of the rounded more developed ones. In some cases there was only a narrow border of red outside the brown. (Plate II, No. IV). The texture of these spots was decidedly leathery and hard and in many cases the surface was slightly depressed. Often the roundness of the apple would be changed, showing that there had been a stoppage in the growth of the cells on that side. It is possible that these irregular spots co-

alesce from the older stages.

The outline of the spots in the later stages became definite, usually round (Plate III). The color was then almost black (Plate II, No. V). Outside of this black portion was a brilliant red. This was brightest at the border and radiated over a large part of the apple, sometimes covering half of it, becoming less and less bright, and usually ending in a mottling or striping.

The spot varies from two millimeters to about three centimeters in diameter (Table I). The progress of the fungus is very slow. Two apples were marked on the twelfth of November, and on the twelfth of March there was not more than 2 mm. of growth on the blackened part of one, the reddened portion apparently remaining the same, and no change at all on the other. The growth probably takes place in the orchard as the fruit develops.

Structure in Cross Section

The fungus in the deepest spot observed penetrated only about 3 mm. deep. The average was from 1 to 2 mm. Plate I, Figs. 1, 2, 3, 4, 5, give an idea of the appearance of the spot in cross section. Fig. 1 shows the flattening effect of the fungus. In Fig. 2 the area where the epidermis is darkest and thickest is more sunken. This is probably where the infection began, as the fruit did not develop evenly. Fig. 3 shows only the margins sunken. In Fig. 4 the thick vein in the fruit prevented the fungus developing at that point. A very deep infection is shown in Fig. 5. This was from one of the darkest colored spots.

The growth of the mycelium into the tissue caused the

flesh to turn brown, the cells becoming corky and hard, and they were filled with a large quantity of starch (Plate I, Fig. 6). Starch was not present to such an extent in the healthy cells (Fig. 7). The fungus appeared to be present only in the brown areas. In the very early stages no infected cells were seen, but from such spots Alternaria was isolated. The depth of the diseased tissue varied with the degree of darkness of the epidermis. This tissue was slightly bitter to the taste, and hard to chew as compared with the healthy flesh.

The mycelium of the fungus can plainly be seen in the cells of the infected tissue (Plate I, Fig. 6). Under the very dark portions of the skin the mycelium was abundant, while under the lighter colored areas it was more difficult to find. Under the reddest portion of the skin radiating from the spot, none was apparent, and no culture was produced from this region.

Methods of Culture

In isolating the fungus from the apples all possible precautions were taken in order to avoid contaminations. Except where otherwise specified the medium used for all cultures was cornmeal agar made according to the following formula:

Cornmeal	50 gms.
Water	1 liter
Agar	1.5 %

This was thoroughly sterilized in the autoclave for one-half hour at fifteen pounds pressure. The tubes and dishes were

heated to 160 degrees Centigrade or above in a hot air oven for at least fifteen minutes. Before pouring the plates, the plugs of the tubes were flamed, and also the ends of the tubes. The pouring was done in a steamed room.

Before any cultures were made from the tissue of the apples the surface, which had been washed in water, and dried with a clean towel, was wiped with sterilized cotton saturated with absolute alcohol. The needles were flamed before the isolations were made.

The Fungus

As is shown in Table I, all but two of the isolations attempted gave Alternaria. The cultures were all alike and originated from the point where the needle was put in the agar. From the number of isolations and the uniformity of the results there would seem to be little doubt that this is the organism which causes the spot, although inoculations on healthy apples did not produce the same spot. It is probable that in the orchard the fungus gains entrance while the fruit is young, and develops more or less with it. In the laboratory no results were obtained without breaking the skin, and when the inoculations were made under the surface a soft rot was produced without any darkening of the skin of the fruit. Perhaps the mature condition of the fruit on which inoculations were made allowed the rot to be soft.

The Alternaria causing this spot is not morphologically different from many other Alternarias which grow on a great many hosts. Mr. Elliott of this laboratory determined it as

Alternaria tenuis, variety X. Two other varieties, one rotting the core of the apple, and one growing on the surface of the seed and not rotting the fruit, while they are alike under the microscope and the colonies look the same, do not behave in the same way when inoculated on the fruit. The one causing this spot produces a rather rapid rot, the one from the core a slower growing rot, and the one from the seed no rot at all. Several cultures obtained from Mr. Elliott indistinguishable from these, produced no rot on the apple. One, however, caused a spot not unlike the one above described. It developed very slowly, and made the skin dark, hard, and sunken. This was A. fasciculata, of the A. tenuis group.

Cultures

In culture the fungus produces a colony which grows very rapidly. There is generally abundant white or grayish, cottony, aerial mycelium (Plate 4, Fig. II) which covers a very large number of spores produced from the internal mycelium. The under side of the culture is dark, with numerous, more or less distinct, concentric rings. The colony is indistinguishable from those of a large number of Alternarias.

The effect of different media upon the fungus is shown in Table II. Cornmeal gave a darker colored colony than bean (Plate 4, Figs. 2, 3) and a rather more abundant growth. The orange juice in the cornmeal seemed to retard the growth for a time, but not permanently. Under unfavorable conditions as in E. and F the fungus spread over a greater area but was much lighter

TABLE 2

Culture	Medium	March 2*	March 3
A	Cornmeal	Growth begun	Colony 1.3 cm. Dark colored. A few spores
B	Cornmeal and Orange juice	No growth	Slight growth. No spores.
C	Bean	No growth	Colony 1 x 1.5 cm Light colored. No spores.
D	Synthetic** No Asparagin	Growth begun	Colony 1 x 1.5 cm light colored. No spores.
E	Synthetic Two parts Asparagin	Growth begun	Colony .5 x 5cm. Only slight advance. No spore
F	Synthetic without glucose	No growth	Very slight sign of light colored mycelium. No spores.

*Cultures started March 1.

**Formula for Synthetic Media

1 liter of water	.50 gm. $MgSO_4$
1.36 gm. KH_2PO_4	5.00 gm. Glucose
1.06 gm. Na_2CO_3	1.00 gm. Asparagin
155 gm. agar	

TABLE 2

March 6	March 8
Colony 3.5cm. Mycelium dark brown below, white flucculent aerial mycelium on surface. Spores abundant.	Colony 4.0 cm. Abundant growth of greenish white aerial mycelium. Spores abundant.
Colony 1.5cm. Bluish growth of aerial mycelium, not as high as in (a). Spores abundant.	Colony 4.0cm. Abundant growth as in (A) but color of colony more bluish.
Colony 2.5 x 3cm. Mycelium light straw colored, not as profuse as in (A) and (B) Spores abundant.	Colony 5.0 cm. Still light colored. Aerial my celium as in(A) and (B). Spores abundant.
Colony 2.5 x 3cm. A little more growth than (C), lighter than (A) or (B), not as many spores.	Colony 4.5 cm. Still darker than (C), not as much aerial mycelium Spores still more abundant.
Colony 3 cm. x 3 cm. Very poor growth, weak, light colored little aerial mycelium.	Colony 5 cm. Very light straw colored. Spread over more surface but weak growth. Spores few.
Colony 2cm. Mycelium all light colored. A few chains of nearly hyaline spores.	Colony as before. A few more chains of spores, darker in color.

in color and less abundant in growth, and formation of spores, (Plate 4, Fig. 4). Lack of glucose had a very marked effect.

The Conidia

The conidia vary in length from 31 to 57 microns, the average being 43. These measurements do not include the beak. The latter varies greatly. The end beak of a chain may be very long, while the spores within the chain may have almost none.

TABLE 3

Spore Measurements

Length	Width	Length of Beak	
31^{1}	9^{1}	2^{2}	18^{5}
36^{7}	11^{1}	5^{1}	21^{1}
41^{3}	13^{1}	7^{1}	22^{1}
43^{1}	14^{11}	9^{1}	23^{1}
45^{5}	16^{2}	11^{3}	25^{1}
54^{4}	18^{2}	14^{2}	30^{1}
57^{1}	22^{1}	16^{1}	38^{1}
Av. 43	Av. 16	Av. 13	

Note:- All measurements in microns.

Sometimes spores are produced on the end of a long beak (Plate I, Fig. 10). The width of the spores was from 9 to 22 microns, the average being 13. Thus the spores are about three times as long as wide, not including the beak. They are almost always

pointed and regular in shape when just mature (Plate I, Figs. 3, 8, 9) but when they are old they become wider, misshapen, the beak disappears, and the cells become constricted (Plate II, Fig. 11). The normal spores are dark in color and have from four to eight transverse septae. In the young or just mature spores there are few longitudinal septae, but when they become old and constricted there are many. The spores are borne in great numbers and are almost without exception in chains, the number in a chain averaging about twelve. From one or more of these spores a side chain may be produced, having as many more (Plate I, Fig. 8). On synthetic media containing no glucose, the chains are shorter and the spores smaller.

Conidiophores

The conidiophores vary in length from 100 microns or more to less than 10. They are invariably dark colored and in most cases the cell next the first spore is enlarged slightly, (Plate I, Fig. 8). The width is about the same as that of the mycelium. The septations are often more numerous than in the mycelium.

Mycelium

The mycelium is dark colored when well developed, but light when young. It varies from 3 to 9 microns in width. When the mycelium branches a new cell is formed at one side, making almost a right angle, (Plate I, Fig. 14). The aerial mycelium is light colored, much finer, the septations are further apart, and little nodules often appear either at the ends or at the

points between (Plate 1, Fig. 15).

Literature

There are only a few references in literature to Alternaria as an enemy of the apple. Morse[1] mentions the leaves as being attacked. Stakman and Rose[2] of Wisconsin describe an Alternaria on the fruit of the Wealthy apple. It causes a small brown spot in some ways resembling those above described. Personal communication with these authors, however, proved it to be different. The spot they write of is much smaller, different in color, and has a more regular outline. They were able to produce typical spots when the organism was inoculated on the fruit, which I have not been able to accomplish.

Longyear[3] of Colorado has found Alternaria causing a blossom and core rot which enters in the stamens and stigmas and usually occurs in storage. These are both soft rots. Cook and Martin[4] found Alternaria in the Jonathan spot, and consider it to be the cause. They produced typical spots with the isolated organism. However, Scott[5] considers that the Jonathan Spot is due possibly to arsenical poisons, and Norton[6] ascribes it to gases from the cooling plants. Brooks, Fisher, and Cooley[7] found Alternaria in apples in storage.

It is possible that all these diseases, including the one above described, are caused by the same organism, a variety of A. tenuis, which has under different environmental conditions become changed. It is a question whether their behaving differently when inoculated on apples is sufficient reason for their

being considered as a distinct species.

Conclusions

More work will have to be done before it can be determined definitely how this spot is formed. Although Alternaria has been found in every case, it may or may not be the direct cause. The time and method of entering the fruit will have to be determined in the orchard. Nothing certain can be said with regard to it until a typical spot has been reproduced by inoculation, but the presence of the Alternaria in the spots seems significant.

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APPENDIX

Since there is a marked resemblance between the spot as described in this and that attributed to Lime Sulphur injury as described by the Illinois Experiment Station (Bulletin 185), I wrote to Mr. E. W. Allen of Harristown, Illinois, from whose orchard the fruit came, to learn the history of the fruit as it developed, especially with regard to the spray used. Mr. Allen writes the following:

"I went over my trees and picked off all apples showing the spots, which amounted to a barrel. There was very little trouble after that. Two of the apples you have were from an orchard one mile away where no spray had been used.

"As to fungicides, I did dormant spraying 8 gallons Commercial Lime Sulphur to 50 gallons of water, then just as petals were about all off I used one gallon of Lime Sulphur to 50 of water and two pounds of Arsenate of Lead paste to 50 gallons of water. Then in ten days used the same spray again, and that was all I did that season.

"I had a fine apple crop but it looked for a while like the trouble would ruin my crop. The first sign would be a little red spot, then it would enlarge to the size of a 5 cent piece, then get brown to a black."

EXPLANATION OF PLATE ONE

Figs. 1, 2, 3, 4, 5, - Diagrams of cross sections of the apple showing depth the fungus penetrates the tissue.

Fig. 6 - Tissue of diseased portion, showing mycelium and starch grains.

Fig. 7 - Tissue of healthy portion showing cells - no starch grains.

Fig. 8 - Chain of conidia from conidiophores; also a chain developing from a spore in the chain. Conidia with longitudinal septations.

Figs. 9, 9a, 9b - Spores having different lengths of beaks.

Fig. 10 - Spore with small spore developing from end of a long beak.

Fig. 11 - Old spore showing round instead of angular cells.

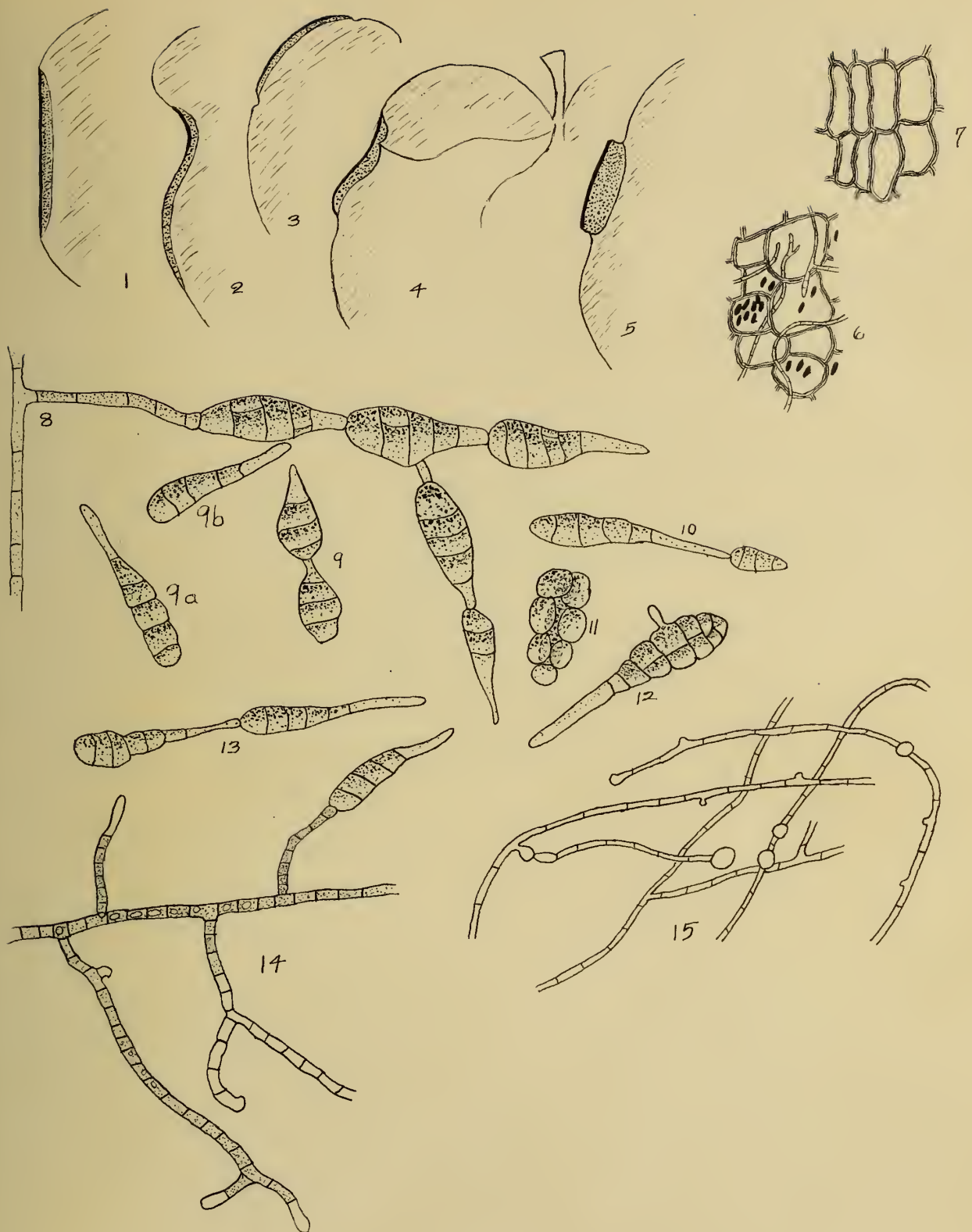
Fig. 12 - Mature spore beginning to germinate.

Fig. 13 - Typical spores with long beaks.

Fig. 14 - Mycelium from cornmeal agar showing the mature part dark and the new light. Conidiophores dark.

Fig. 15 - Aerial mycelium, showing comparative size as compared with internal mycelium. Note the enlarged cells or nodules.

Plate I



EXPLANATION OF PLATE II

No. I. Very early stage of infection.

No. II. Stage a little later than No. I.

No. III. Irregular spots of dark brown infected tissue

No. IV. Late stage.

No. V. Last stage, tissue almost black.

No. I

No. II

No. III

No. IV

No. V

EXPLANATION OF PLATE III

No. 1. Apples showing three sizes of spots - natural size. Upper right hand apple marked both around darkened part and around reddened part. There was no progress of the spot beyound these lines in four months.

No. II. Left hand apple showing irregular shaped spot. Right hand one showing depression around edge of spot.

** **

I

II

EXPLANATION OF PLATE IV

No. I. Cross section of two infected fruits. On the upper side of left hand fruit may be seen the infected portion - about 2 mm. in depth and 10 mm. wide. On the lower part of the right hand one may be seen the darkened tissue about 3 mm. deep and 2 cm. wide. This shows the depressed edges.

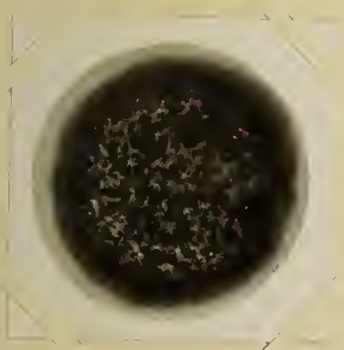
No. 2. Culture on cornmeal agar.

No. 3. Culture on bean agar.

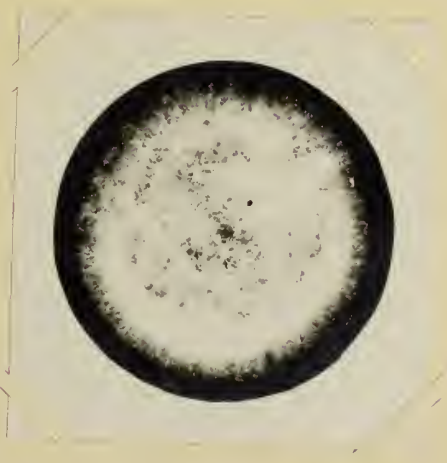
No. 4. Culture on synthetic with no glucose.

Plate IV

I



II



III

IV

AN APPLE ROT CAUSED BY GLIOCLADIUM VIRIDE

Introduction

The fungus upon which this paper is based was found on several plates which were made while isolating fungi from diseased apples. When inoculated on healthy fruit it produced a rot.

The Fungus

The mycelium of this fungus is hyaline, creeping, septate, branching, and varies from 1.8 to 3.6 microns in width (Fig. 1).

The conidiophores are formed as in Penicillium. They grow erect from the internal mycelium, branching once to many times (Figs. 7, 8, 9). They are hyaline, septate, and can only be differentiated from the aerial mycelium by the profuse branching. These branches become brushlike as in Penicillium (Fig. 13). Matruchot[1] describes the branches of the conidiophores as budding just below a septa and growing up parallel to the main axis (Fig. 15).

The conidia are borne in great numbers on the ends of the conidiophores. According to Matruchot they are borne in chains, but remain so only for a very short time. Examination of all stages of this fungus has not shown chains of spores, but rather a new spore borne from the same point as the old one and pushing the older one aside (Fig. 6). Almost immediately the spores produce externally a gelatinous substance which glues them tightly together in masses. Matruchot describes the sticky coating as a white milky substance. The spores upon one conidiophore may be attached to those of a whole cluster forming a large mass which

1. Matruchot, L.; Bul. Soc. Myc. 1893, p. 246.

may vary greatly in size and shape (Fig. 2). These masses are at first greenish but later almost invariably become black.

The individual spores are elliptical to ovate in shape and may or may not be pointed at the ends (Fig. 3). In size they vary from 5.4 by 2.7 microns to 9 by 3 microns. They are dark in color. Germination is rapid; spores in a hanging drop of distilled water showed a germination of about 50 % in 9 hours (Fig. 4). In a hanging drop of cornmeal agar there was considerable growth in 12 hours (Fig. 5).

Cultures

On cornmeal agar this fungus produced a very characteristic colony (See photo). As the colony was developing there was an abundance of white, flucculent, aerial mycelium. This more or less disappeared when the spores began to form. The spore masses were generally produced in concentric rings which were distinct. These rings were white when first produced but they darken, sometimes becoming green, and then black. There seemed to be some variation in color even when grown on the same medium and under the same conditions. The spore clusters were very irregular in size and shape (Fig. 2) depending upon the number held together in the gelatinous substance. The older the colony the larger the clusters were. A tube four months old showed a cluster 1 cm. long and 2 cm. wide.

No difference could be noticed in the size or shape of the spores or the mycelium on the different media used, but the number of spores produced, the size of the spore clusters, and the abundance of the mycelium varied.(Table I). When double the

TABLE I

Medium	Size of Colony on: Apr.19	Apr.24.	May 3	Description of Culture
Cornmeal	1.0 cm.	2.5 cm.	6.0 cm.	Very good colony, light colored with concentric rings of either black or greenish masses of spores.
Bean	1.0 cm.	3.0 cm.	7.0 cm.	Like above but more spores and slightly darker color.
Synthetic	1.0 cm.	2.5 cm.	6.0 cm.	As above but no concentric rings
Synthetic Asparagin doubled	1.5 cm.	3.0 cm.	5.0 cm.	Abundant growth of mycelium and spores, concentric rings more or less distinct.
Synthetic No asparagin	-	-	5.0 cm.	Colony spread over a large surface but very poor growth. Spores few.
Synthetic Glucose doubled	1.0 cm.	-	6.0 cm.	Abundant growth of mycelium but spores few and not in rings.
Synthetic No glucose	1.0 cm.	-	5.0 cm.	Very light color, very little mycelium and but few clusters of spores.

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amount of asparagin was in the synthetic medium used the spores and mycelium were abundant. In media with double the amount of glucose the growth of mycelium was large, but that of spores few. On synthetic media without asparagin the culture was weak and spread over a large surface. The same was true when no glucose was present. In the last two media the spores, when produced, were in a few large, apparently normal clusters instead of in many small ones distributed over the colony. On plain agar the development was weak and growth ceased entirely after the colony reached a size of about 2 cm.

Inoculation

When inoculated on healthy apples the fungus produced a characteristic spot. The development was slow. About two weeks time was necessary to show any distinct spot. In many cases no infection took place. The spot when produced was light brown with a distinct margin which was slightly darker than the rest of the diseased portion. After about a month the spot was sunken and had not developed over 1 cm. in diameter. The surface was slightly hardened.

In cross section the spot showed in many cases a diseased portion of much larger area than could be seen superficially. The fungus had, in one case for example, penetrated almost into the core of the apple in three weeks. It made a rather soft, spongy rot - not as soft, however, as that of Penicillium. While the surface spot in this case was only .5 cm. in diameter, the tissue below was rotted 2 cm. deep and 1 cm. in either direction from the spot. In other cases the rot was not so rapid - penetrating

only about .5 to 1 cm. with a surface spot of .5 cm. after two weeks. No observations have been made upon fruit which had been inoculated more than a month.

The Genus

The genus Gliocladium was described by Corda, the type species being G. penicillioides.[1] According to Saccardo the genus may be described as having the sterile hyphae creeping, the fertile hyphae growing erect, simple, septate, and forming upon branching a brush-like apparatus upon which the conidia are borne. The conidia are at first catenulate but later are enveloped by a mucilaginous substance into a little head. It is only in the presence of this sticky coat on the spores of the Gliocladium that Gliocladium and Penicillium differ essentially. There are fourteen species given in the Sylloge Fungorum, most of which occur on decaying fungi or other dead organic matter. None of them seems to be of any economic importance except G. agaricinum Corda and Mass. which arrests growth and breaks the pilei of mushrooms.

The Species

The fungus was submitted to Dr. Chas. Thom, who said: "I have examined the culture and find a form which I have studied several times during the last ten years. Thus far, I have called it "Gliocladium viride Matr." The lot to which this belongs needs someone's time and attention, but this one runs close enough to the form named above to forbid separate nomenclature, unless for reasons based on a larger volume of critical study than I have thus far been able to give it." An article by Matruchot[2] gives

1. Corda, A. J. C.. Ic. fung. IV, p. 30.
2. Saccardo, P. A., Syll. II, p. 594.

fully the development of the species. There seem to be some differences between the fungus as described by him and the material at hand (Table II). Further study may justify describing it as a new species. Neither the variation in spore measurement nor the differences in shape of the spores alone would be sufficient grounds for separating it. But G. viride is described as having at first catenulate spores. Examination of a large number of conidiophores with spores varying in number from 1 to 3 or 4 has never shown any to be so. They seem rather to form on the ends of a hyphal thread, a new spore developing at the same point and pushing the old one aside (Fig. 6).

Another difference seems to be in the development of the mycelium. G. viride forms a branch just below a septa which grows up parallel to the main axis, this in turn branching in the same manner. From just above a septa at the same time another branch or series of branches is formed which grows down to the medium parallel to the main axis but having positive geotropism (Fig. 15). This structure has not been observed in my material. In all sections studied the main hyphal thread came directly from the medium and produced the conidiophore unsupported by aerial branches.

But a still more noticeable difference is in the color of the masses of conidia. G. viride Matr. is described as green. In most cases the specimens in my cultures are black when mature, tho some variation was observed in the young colonies.

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TABLE II

COMPARISON OF G. VIRIDE MATR. WITH THE SPECIES UNDER DISCUSSION

	Gliocladium viride	Gliocladium sp.
Spore measurement	3 - 6 x 2 - 3	5.4-9 x 2.7 - 3.6
Spore shape	Oval and pointed	Elliptical to ovate, sometimes pointed.
Spore catenulation	Catenulate at first	Never catenulate in large number observed.
Development of the mycelium	Brush-like structure of mycelium grows both up and down	Brush-like mycelium grows only up

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EXPLANATION OF PLATE

Fig. 1 - Mycelium showing methods of branching.

Fig. 2 - Spore masses showing irregularity in size and shape (low power).

Fig. 3 - Spores.

Fig. 4,5-Germinating spores.

Fig. 6, 7 - Conidiophores with the developing conidia.

Fig. 8, 9 - Conidiophores.

Fig. 10, 11, 12. 13 - Spore masses held together by the gelatinous substance.

Fig. 14 - Conidiophores with spores before agglutination.

Fig. 15 - Diagrammatic representation of growth of mycelium as described by Matruchot.

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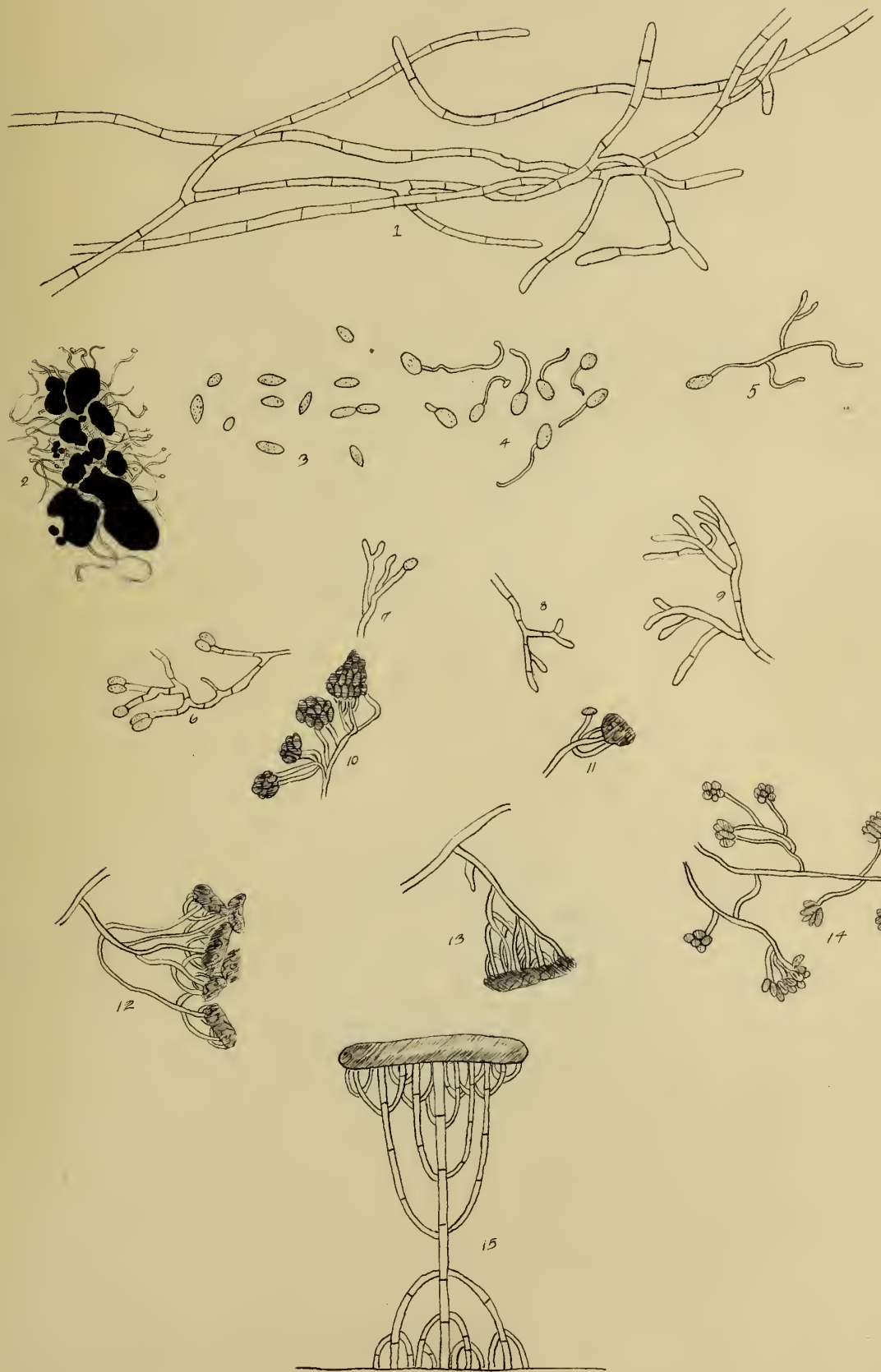
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Colonies of Gliocladium viride

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